rejected F344 rat skin grafts in less than 9 days. Furthermore, these same animals rejected the third party allogeneic B10.D2 skin grafts with an MST identical to that of normal B10 controls. This result has subsequently been confirmed in two additional groups of mice. In one of these groups, an additional skin graft was performed from a WF rat, a rat strain differing from the F344 at both major and minor histocompatibility loci[11]. The WF skin grafts were all rejected promptly, while the F344 skin grafts again showed the same prolonged survival.

Numerous previous studies[7,12,13] have shown that elimination of mature T cells from donor bone marrow inocula can lead to long-term survival of fully allogeneic bone marrow chimaeras. However, the survival of such animals is generally not as good as that of syngeneically reconstituted controls, and the health of these animals is compromised, probably as the result of partial immunoincompetence[5,6]. The model for fully xenogeneic chimaeras[14] has been less extensively studied than that of allogeneic chimaeras. In our experience, such animals show prolonged survival over irradiation controls, but all succumb within a few weeks following reconstitution. It is not clear whether the death of these animals is due to a chronic graft–versus-host disease, or to infectious complications secondary to partial immunoincompetence.

In contrast, the studies presented here indicate that reconstitution with mixtures of syngeneic bone marrow and either allogeneic or xenogeneic marrow leads to long-term survival of recipients equivalent to that of syngeneically reconstituted animals. The animals have remained healthy and have shown no evidence of infections or of graft–versus-host disease for longer than 3 months.

Typing of mixed allogeneically reconstituted animals at 2–3 months following reconstitution showed them to be truly mixed chimaeras, bearing variable percentages of syngeneic and allogeneic lymphoid cells in their peripheral circulation. Such animals are immunocompetent[13] and specifically tolerant to donor skin grafts. In mice reconstituted with mixed syngeneic and xenogeneic bone marrow, we have found little evidence for persistence of the xenogeneic lymphoid compartment. However, these animals also appear to be specifically hyporeactive to subsequent skin xenografts. They may be more similar to neonatally induced allochimaeras[15], in which only very small numbers of persistent donor-type cells can be found[16]. Since the survival of skin grafts is frequently more difficult to prolong than that of most other foreign tissues[17], it seems likely that animals manifesting this level of hyporeactivity to skin grafts would be highly tolerant to xenografts of kidneys, hearts and other solid organs.

These data therefore indicate that reconstitution of lethally irradiated mice with mixtures of syngeneic and either allogeneic or xenogeneic bone marrow leads to the induction of specific immune unresponsiveness to subsequent donor skin grafts. The mechanism of this specific unresponsiveness may not be the same in these mixed allogeneic and xenogeneic models, and further mechanistic studies both *in vitro* and *in vivo* are presently in progress. Both types of reconstitution offer models which may be directly applicable to organ grafting in other species, including man.

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1. Rapaport, F. T. *et al. Transplantn Proc.* **9**, 891–894 (1977).
2. Slavin, S., Strober, S., Fuks, Z. & Kaplan, S. *J. exp. Med.* **146**, 34–48 (1977).
3. Dittmer, J. & Bennett, M. *Molec. cell. Biochem.* **21**, 83–94 (1978).
4. Myburgh, J. A., Smit, J. A., Browde, S. & Hill, R. R. H. *Transplantation* **29**, 401–404 (1980).
5. Zinkernagel, R. M., Althage, A., Callahan, G. & Welsh, R. M. *J. Immun.* **124**, 2356–2365 (1980).
6. Rayfield, L. S. & Brent, L. *Transplantation* **36**, 183–189 (1983).
7. Auchincloss, H. Jr & Sachs, D. H. *Transplantation* **36**, 436–441 (1983).
8. Arn, J. S., Riordan, S. E., Pearson, D. & Sachs, D. H. *J. immun. Meth.* **55**, 141–153 (1982).
9. Billingham, R. E. in *Transplantation of Tissues and Cells* (eds Billingham, R. E. & Silvers, W. K.) (Wistar Institute, Philadelphia, 1961).
10. Sharrow, S. O., Mathieson, B. J. & Singer, A. *J. Immun.* **126**, 1327–1335 (1981).
11. Gasser, D. L. *Adv. Immun.* **25**, 93–139 (1977).
12. Krown, S. E., Coico, R., Scheid, M. P., Fernandez, G. & Good, R. A. *Clin. immun. Immunopath.* **19**, 268–283 (1981).
13. Singer, A., Hathcock, K. S. & Hodes, R. J. *J. exp. Med.* **153**, 1286–1301 (1981).
14. Muller-Ruchholtz, W., Muller Hermelink, H. K. & Wottge, H. U. *Transplantn Proc.* **11**, 517–521 (1979).
15. Billingham, R. E., Brent, L. & Medawar, P. B. *Nature* **172**, 603 (1953).
16. Streilein, J. W. & Klein, J. *J. Immun.* **119**, 2147–2150 (1977).
17. Boyse, E. A., Lance, E. M., Carswell, E. A., Cooper, S. & Old, L. J. *Nature* **227**, 901–903 (1970).

Genetic evidence of X–Y interchange in a human XX male

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Of the hypotheses put forward to explain why occasional individuals with two X chromosomes are nonetheless male, the one that has attracted most attention is the possibility[1] that one of the X chromosomes has obtained a small piece of Y chromosome which is sufficient to produce 'maleness'. This hypothesis was based primarily on the observation that in two families with XX males[2–4] both fathers were Xg(a+) and both probands Xg(a−). (*Xg* shows X-linked dominant inheritance.) This theory holds that an anomalous X–Y interchange at meiosis in the father resulted in the paternal X chromosome's losing the *Xg* gene and acquiring a male-determining gene from the Y chromosome. While, for example, the frequencies of Xg phenotypes among XX males[5,6] and the cytogenetic observation of a structural abnormality in one X[7,8] are compatible with this hypothesis, direct evidence of it is lacking. Here we describe an XX male who expresses his father's allele for 12E7, a Y-linked marker, but fails to express his father's allele for Xg, an X-linked marker. These findings strongly suggest that anomalous X–Y interchange occurred in this case and perhaps in that of many other XX males. We suggest that a male-determining gene on the Y has also been translocated to the X and caused maleness in the proband. These results are discussed in the light of current models of X–Y chromosomal homology.

A boy born in 1980 had slightly dysmorphic facial features but was otherwise normal. He has remained in good health but his physical growth is greatly retarded (at 2 yr and 3 months old, height = 75 cm, weight = 7.4 kg). His height development is well below the 2.5 percentile for boys and even for girls. Clinical and laboratory investigations had failed to disclose anything abnormal to account for the retarded growth until a karyotype showed him to be 46, XX; there was no evidence of mosaicism in the two tissues karyotyped (lymphocytes and skin fibroblasts). The parents (born in 1953) are non-consanguineous. All four grandparents are alive. The proband has a brother born in 1978 and a sister born in 1983. All family members are healthy.

All family members (Fig. 1) were tested for the following blood groups: ABO, MNSs, P_1, Rh, Lu^a, Kell, Lewis, Duffy, Kidd, Dombrock and Co^b. That II-1 is indeed the father of III-2 is supported by the inheritance of these markers (data not shown). Figure 1 shows the segregation of the blood group Xg, the antigen 12E7, and the polymorphic X–Y homologous DNA locus designated *DXYS1* (ref. 9) in the members tested. Both parents, II-1 and II-3, are Xg(a+); the mother is heterozygous Xg^aXg as her father, I-3, is Xg(a−). Both the proband, III-2, and his brother, III-1, are Xg(a−) so they have received the maternal *Xg* gene. All Xg(a+) individuals express high levels

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of 12E7 (ref. 10). However, in families with Xg(a−) members, the quantitative polymorphism of 12E7 expression shows Y-linked inheritance[11]. For example, 77 Xg(a−) high-level 12E7 males (first tested member of sibship) have 110 Xg(a−) younger (or tested later) brothers of whom 107 have high-level 12E7 and 3 have low-level 12E7 expression; 18 Xg(a−) low-level 12E7 males have 19 Xg(a−) younger brothers of whom 17 have low-level 12E7 and 2 have high-level 12E7 expression. These comparisons give $\chi^2 = 20.51$ and 15.81 respectively if 12E7 were not Y-controlled. The five exceptional brothers could be the result of X–Y interchange, or of difficulties in testing travelled red cells, or of a different father (the father of only one of the exceptions has been tested)[11].

In the family reported here, both the paternal uncle, II-2, and elder brother, III-1, are Xg(a−) and high expressors of 12E7, suggesting that the Y chromosome in the paternal family carries Yg^a. (The Y-linked *12E7* locus has been designated *Yg* on the basis of its hypothesized homology to *Xg*[10]. The allele for high expression of 12E7 has been designated Yg^a.) The maternal grandfather, I-3, is Xg(a−) low 12E7 and, therefore, *XgYg*.

The proband does not carry the paternal Xg^a allele but has received a gene producing high 12E7 expression. As this could not have come from the mother, the most straightforward explanation is an interchange of the *Xg* and *Yg* loci in paternal meiosis. As a consequence of this, the X chromosome of paternal origin has lost its Xg^a but acquired Yg^a. The presence of testes and male differentiation in the proband is probably the consequence of a sex-determining gene having been exchanged as well. Perhaps a male-determining gene normally located in proximity to *Yg* on the Y chromosome has been translocated to the X. Alternatively, an X-borne gene involved in sex determination may have been lost or inactivated.

Our interpretation assumes that one of the proband's X chromosomes is indeed paternal. The *Taq*I restriction fragment length polymorphism at the *DXYS1* locus shows normal segregation of the Y-specific 15-kilobase (kb) fragment with the Y chromosome in the father, II-1, and brother, III-1 (Fig. 1). The proband has a double dose of the X-specific 11-kb allele (one from the father and one from the mother). This is not formal proof of the paternal derivation of one X, as it is also consistent with the proband having two copies of the maternal X carrying the 11-kb allele (and *Xg*). However, the latter possibility is inconsistent with the 12E7 data. The segregation of eight additional X-linked DNA polymorphisms was examined in this family but none of the polymorphisms proved to be informative with respect to a paternal contribution (data not shown).

It has been shown recently that many XX males have one paternal and one maternal X chromosome. When the segregation of the *DXYS1* alleles was tested in six families, four were informative. In each of these four cases the XX male proband was heterozygous[11,12] while the mother was homozygous for one of the alleles and the father hemizygous for the other (D.P. and A. de la C., unpublished results). This supports the notion that one X is paternal in many, if not most, XX males.

The proposed interchange explains three findings (loss of Xg^a, acquisition of Yg^a, maleness) as the consequence of one event. The alternative—non-disjunction at maternal meiosis plus non-disjunction at the first paternal meiotic division or zygotic loss of the Y—does explain the chromosomal and Xg data but fails to explain the level of 12E7 expression and the maleness of the proband. Hence, the interchange mechanism must be considered the most likely one.

At present we do not know what proportion of XX males arises by the type of interchange that occurred in this case. That it may be common is suggested by the fact that at least nine families are known in which the XX male proband apparently did not inherit his father's *Xg* allele[6]. Furthermore, the frequency of Xg phenotypes in XX males is close to that of XY males and is significantly different from that of females (R. Sanger and P.A.T., unpublished observations on 94 northern European XX males).

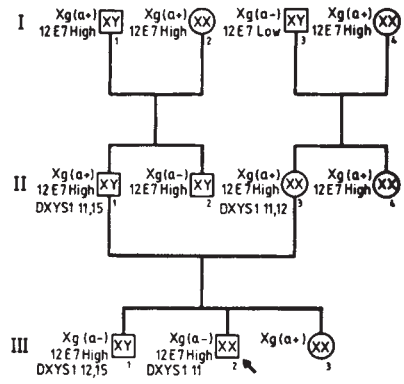

Fig. 1 Pedigree of the family showing the sex chromosome constitution and segregation of the blood group Xg, the antigen 12E7, and the X–Y homologous DNA locus *DXYS1*.

In contrast, at least five families are known in which the XX male proband did inherit his father's *Xg* allele[6]. These cases either arose by another mechanism or the interchange involved different regions of those chromosomes. Light and electron microscopic evidence[12,13] indicates that terminal portions of the short arms of the human X and Y chromosomes normally pair at male meiosis. It has been suggested that crossing-over within this 'pairing region' occurs as a normal event[1,14–16], but there is no genetic evidence for this. Here we have described a clearly anomalous meiotic interchange of normally X-linked and Y-linked markers. Of note is the fact that the *Xg* gene maps to the 'pairing region' on the X[17] while a *12E7* structural gene maps to the euchromatic portion of the Y[18], which includes the pairing region. Thus, the X–Y interchange reported here may be an example of a common event, but one which is abnormally proximal to the centromere.

The aetiology of XX males is probably heterogeneous. The underlying mechanisms may include errors of X-chromosome inactivation, mutations in autosomal or X-chromosomal genes, and X–Y interchange (probably with different breakpoints in different cases). A somewhat analogous finding is that the sex reversal mutation in the mouse (*sxr*) is now known to be due to a complicated Y–X exchange[19,20].

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1. Ferguson-Smith, M. A. *Lancet* **ii,** 475–476 (1966).
2. de la Chapelle, A., Hortling, H., Niemi, M. & Wennström, J. *Acta med. scand. Suppl.* **412,** 25–38 (1964).
3. de la Chapelle, A., Hortling, H., Wennström, J., Niemi, M. & Johansson, C.-J. *Acta endocr. Suppl.* **100,** 90 (1965).
4. de la Chapelle, A., Similä, S., Lanning, M., Kontturi, M. & Johansson, C.-J. *Hum. Genet.* **11,** 286–294 (1971).
5. Race, R. & Sanger, R. *Blood Groups in Man* 6th edn (Blackwell, Oxford, 1975).
6. de la Chapelle, A. *Hum. Genet.* **58,** 105–116 (1981).
7. Evans, H. J., Buckton, K. E., Spowart, G. & Carothers, A. D. *Hum. Genet.* **49,** 11–31 (1979).
8. Magenis, R. R. *et al. Hum. Genet.* **62,** 271–276 (1982).
9. Page, D. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 5352–5356 (1982).
10. Goodfellow, P. N. & Tippett, P. *Nature* **289,** 404–405 (1981).
11. Tippett, P., Shaw, M.-A., Daniels, G. L. & Green, C. A. *Human Gene Mapping* Vol. 7 (in the press).
12. Pearson, P. C. & Bobrow, M. *Nature* **226,** 959–961 (1970).
13. Moses, M. J., Counce, S. J. & Paulson, D. F. *Science* **187,** 363–365 (1975).
14. Polani, P. E. in *Mechanisms of Sex Differentiation in Animals and Man* (eds Austin, C. R. & Edwards, R. G.) 465–488 (Academic, London, 1981).
15. Polani, P. E. *Hum. Genet.* **60,** 207–211 (1982).
16. Burgoyne, P. S. *Hum. Genet.* **61,** 85–90 (1982).
17. Ferguson-Smith, M. A., Sanger, R., Tippett, P., Aitken, D. A. & Boyd, E. *Cytogenet. Cell Genet.* **32,** 273–274 (1982).
18. Goodfellow, P. *et al. Nature* **302,** 346–349 (1983).
19. Singh, L. & Jones, K. W. *Cell* **28,** 205–216 (1982).
20. Evans, E. P., Burtenshaw, M. D. & Cattanach, B. M. *Nature* **300,** 443–445 (1982).